

The following article appeared in Journal of Experimental Botany 62(15): 5581-5594 (2011); and may be found at: <https://doi.org/10.1093/jxb/err240>

This is an open access article under the Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0) license <https://creativecommons.org/licenses/by-nc/4.0/>

RESEARCH PAPER

Allometry of root branching and its relationship to root morphological and functional traits in three range grasses

J. Tulio Arredondo^{1,*} and Douglas A. Johnson²

¹ División de Ciencias Ambientales-Instituto Potosino de Investigación Científica y Tecnológica, Camino presa San Jose 2055, Lomas 4ta sección, 78216, San Luis Potosí, S.L.P., México

² USDA-ARS Forage and Range Research Laboratory, Utah State University, Logan, UT 84322-6300, USA

* To whom correspondence should be addressed. E-mail: tulio@ipicyt.edu.mx

Received 17 March 2011; Revised 24 June 2011; Accepted 13 July 2011

Abstract

The study of proportional relationships between size, shape, and function of part of or the whole organism is traditionally known as allometry. Examination of correlative changes in the size of interbranch distances (IBDs) at different root orders may help to identify root branching rules. Root morphological and functional characteristics in three range grasses {bluebunch wheatgrass [*Pseudoroegneria spicata* (Pursh) Löve], crested wheatgrass [*Agropyron desertorum* (Fisch. ex Link) Schult. × *A. cristatum* (L.) Gaert.], and cheatgrass (*Bromus tectorum* L.)} were examined in response to a soil nutrient gradient. Interbranch distances along the main root axis and the first-order laterals as well as other morphological and allocation root traits were determined. A model of nutrient diffusivity parameterized with root length and root diameter for the three grasses was used to estimate root functional properties (exploitation efficiency and exploitation potential). The results showed a significant negative allometric relationship between the main root axis and first-order lateral IBD ($P \leq 0.05$), but only for bluebunch wheatgrass. The main root axis IBD was positively related to the number and length of roots, estimated exploitation efficiency of second-order roots, and specific root length, and was negatively related to estimated exploitation potential of first-order roots. Conversely, crested wheatgrass and cheatgrass, which rely mainly on root proliferation responses, exhibited fewer allometric relationships. Thus, the results suggested that species such as bluebunch wheatgrass, which display slow root growth and architectural root plasticity rather than opportunistic root proliferation and rapid growth, exhibit correlative allometry between the main axis IBD and morphological, allocation, and functional traits of roots.

Key words: Bluebunch wheatgrass, cheatgrass, crested wheatgrass, exploitation efficiency, exploitation potential, root allometry, root morphology.

Introduction

Branching design in biological structures is hypothesized to occupy space, with a conservative use of materials maximizing functional benefits (Zamir, 1976; Honda and Fisher, 1978; LeFèvre, 1983; Niklas and Kerchner, 1984; Niklas, 1986; Morgan and Cannell, 1988). Above-ground branching patterns, for example, directly affect light capture, water

Abbreviations: ANOVA, analysis of variance; CV, coefficient of variation; D , diffusion coefficient of the ion in the soil; DW, dry weight; EE, exploitation efficiency (volume of soil exploited per unit volume of root); EE_{1st} , exploitation efficiency (volume of soil exploited per unit volume of first-order roots); EE_{2nd} , exploitation efficiency (volume of soil exploited per unit volume of second-order roots); EE_s , exploitation efficiency (volume of soil exploited per unit volume of total root sample); EP, exploitation potential (volume of soil exploited by roots); EP_{1st} , exploitation potential (volume of soil exploited by first-order roots); EP_{2nd} , exploitation potential (volume of soil exploited by second-order roots); EP_s , exploitation potential (volume of soil exploited by total root sample); FO-IBD, first-order root interbranch distance; IBD, interbranch distance; M:V ratio, root mass to root volume ratio; MA-IBD, main root axis interbranch distance; NU, non-uniform nutrient treatment; NUH, non-uniform high-nutrient treatment; NUL, non-uniform low-nutrient treatment; r_{b1} , radius of the branch of first-order roots; r_{dz} , radius of the depletion zone around each root; r_L , average radius for a particular root order; SRL, specific root length; t , time; UH, uniform high-nutrient treatment; UL, uniform low-nutrient treatment; V_{dz} , volume of the nutrient depletion zone; V_{z1} , volume of the nutrient depletion zone for first-order roots; V_{z2} , volume of the nutrient depletion zone for second-order roots.

© 2011 The Author(s).

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/2.5>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

transport, mechanical support, reproduction, wind resistance, and ultimately the competitive relationships among plants (Küppers, 1989). Although similar functional relationships may be observed for different branching designs of root systems, the understanding of branching development and patterns in root systems is still limited (Zhang and Hasenstein, 1999). Root branching plays an important role in the acquisition of soil nutrients and water (Fitter, 1987; Fitter *et al.*, 1991; Berntson GM, 1994), mechanical support and anchorage of plants (Ennos and Fitter, 1991), balance of carbon and minerals (Nielsen *et al.*, 1994), and competition for soil resources between plants (Hodge *et al.*, 1999; Robinson *et al.*, 1999).

Lateral root primordia are initiated in the root pericycle at particular points located regularly along longitudinal rows in the vascular structure of the parent root (Charlton, 1996). Although lateral roots in different rows appear to be distributed randomly, some reports suggest that in some cases lateral root distribution from the root tip is not random (Mallory *et al.*, 1970; Newson *et al.*, 1993; Charlton, 1996). This could mean that spacing between neighbouring root branches (interbranch distance, IBD) is under strict control to facilitate its functional role for a particular root order level (e.g. primary root, first- and/or second-order branches) (Malamy, 2005). These morphological adjustments may also directly or indirectly influence other root traits such as root branch density (number of branches per unit root length), root surface area, root length, root diameter, and others throughout ontogenetic development.

Several studies have already pointed out the existence of mechanisms that control root branching emergence and root elongation. For example, some studies presented evidence that lateral root emergence involves correlative control mechanisms including the localized production and transport of shoot-derived growth regulators (Sachs, 1991; Forde and Walch-Liu, 2009). Accordingly, Zhang and Hasenstein (1999) reported that initiation and elongation of lateral roots in *Lactuca sativa* L. resulted from a balance between the basipetal flux of a cytokinin-like inhibitor derived from the root apex and the acropetal transport of a shoot-derived auxin that promotes lateral root development. In addition, Zhang *et al.* (1999) and Zhang and Forde (2000) identified two pathways by which the ion NO_3^- modulates root branching, one stimulating root elongation and the other inhibiting branching initiation. More recently, Walch-Liu *et al.* (2006) reported that roots of *Arabidopsis thaliana* (L.) Heynh. respond to exogenous L-glutamate, which inhibits primary root growth but stimulates lateral root branching. These mechanisms that act on cell division and development of additional primordia to initiate new lateral branches may occur whenever cells receive appropriate environmental cues (Dubrovsky *et al.*, 2000; Ermel *et al.*, 2000; Malamy, 2005).

The study of the proportional relationships between size, shape, and function of part of or the whole organism is traditionally known as allometry (Gould, 1966; Reiss, 1989)

or scaling analysis (Niklas, 1994). If correlative control mechanisms of root emergence operate at two distinctive root branching order levels (e.g. the main axis and first-order roots) in response to both genetic/physiological and environmental (light, gravity, moisture, touch, and nutrients) controls (Porterfield, 2002), then IBD patterns could emerge (Mallory *et al.*, 1970; Newson *et al.*, 1993; Charlton, 1996). As a consequence, traits such as spacing among root branches, IBD allometric relationships between different root orders, and the allometric relationship of IBD to other root traits arising at different root orders could have adaptive significance and fulfil different functions, including efficient exploitation of soil resources, reduction of interbranch competition, and control of carbon distribution to individual root members.

Occurrence of allometric IBD relationships may depend, however, on inherent species characteristics and habitat conditions (Crick and Grime, 1987; Robinson, 1994). For example, the well-documented root proliferation that generally takes place in some species as a response to soil nutrient patches, which allows for either rapid acquisition of immobile soil resources (Scott Russell and Clarkson, 1976; Caldwell and Richards, 1986) or improved competitive ability (Hodge *et al.*, 1999; Robinson *et al.*, 1999), may impose restrictions on the development of allometric IBD relationships. For instance, proliferation of fine roots deployed into a particular enriched soil site does not appear to require a precise placement of lateral roots but rather rapid initiation and elongation of multiple roots. In contrast, under deprived soil nutrient conditions that favour the improvement of root foraging precision, the emergence of neighbouring roots at a distance that, for example, delays lateral root competition, would appear to be an appropriate strategy (Berntson, 1994).

Hence, considering the inherent response capacity of species to soil nutrient patches through changes in either root proliferation or root architectural plasticity, this study examined three range grasses with contrasting root growth strategies. It was hypothesized (H_1) that species expressing root proliferation responses to soil nutrient enrichment will not exhibit allometry between IBDs of different root order levels. Conversely, species that exhibit architectural root plasticity should be able to develop allometric IBD relationships. Additionally, if allometric IBD relationships at different root orders occur and they have adaptive significance, it was hypothesized (H_2) that IBDs for the main root axis should exhibit allometric relationships to other morphological and allocation traits that impact the functional properties of the root system under particular soil nutrient conditions. Thus, the aim of this study was to examine the occurrence of root IBD allometric relationships in three range grasses that exhibit either root proliferation or root architectural plasticity, and to test whether IBD characteristics are related to functional attributes of root systems [defined as the significance or consequences of a structure, for a given species in a particular environment (Press, 1999)] using a diffusion model.

Materials and methods

Species descriptions

The study included perennial bluebunch wheatgrass [*Pseudoroegneria spicata* (Pursh) Löve, Whitmar cultivar] that is native to western North America; perennial hybrid crested wheatgrass [*Agropyron desertorum* (Fisch. ex Link) Schult. × *A. cristatum* (L.) Gaert., Hycrest cultivar] with its parents having origins in Central Asia, but which are now naturalized in western North America; and cheatgrass (*Bromus tectorum* L.), an exotic annual grass from Central Asia that is widely distributed throughout western North America. These three grasses are widespread in the sagebrush-steppe ecosystem of the Great Basin Region in the western USA. Bluebunch wheatgrass exhibits delayed and less root proliferation in response to nutrient patches than crested wheatgrass (Eissenstat and Caldwell, 1988; Jackson and Caldwell, 1989), yet exhibits considerable plasticity in root architecture (Arredondo and Johnson, 1999). Cheatgrass exhibits both rapid allocation of biomass to roots and root architectural plasticity (i.e. link length; Arredondo and Johnson, 1999).

Experimental setting

Roots from a study (described in Arredondo and Johnson, 1999) that was conducted in a greenhouse at Logan, Utah, USA were re-analysed. Individual 1-week-old seedlings of bluebunch wheatgrass, crested wheatgrass, and cheatgrass were transplanted into 50 cm³ polystyrene cups that had a 1 mm mesh positioned 1 cm below the mouth of the cup. Seedlings were allowed to grow their roots through the mesh in the remaining volume of the cup. After 3 weeks, when seedlings had developed 3–5 fully expanded leaves and 4–6 root axes, they were transferred to sand-filled (particle size <0.1 mm), 10.0 l pots that had two independent compartments divided by an aluminium partition.

Each pot compartment received either a 3.2% (low) or a 32.2% (high) concentration of Rorison nutrient solution (Hewitt, 1966). Pots were given nutrient treatments that were either uniform [both compartments received either high (UH) or low (UL) nutrient concentration] or non-uniform [one compartment with a high and the other with a low nutrient concentration (NU)]. From high to low, total nutrients in the three nutrient treatments varied UH > NU > UL. Sampling for the NU treatment was conducted in both the non-uniform high (NUH) and non-uniform low (NUL) pot compartments. To examine roots from the NU treatment, the number of pots with this treatment was duplicated. The nutrient solution was added to approximate a replenishment rate of ~2.5 l d⁻¹ 100 l⁻¹ of water for both the high- and low-nutrient supply (Hewitt, 1966).

After 31 d, seedling roots exhibiting primarily first- and second-order branches were washed with a fine spray of water. One primary root axis was selected from each compartment, and a 10 cm segment of this root axis was used for detailed morphological analysis. Several studies have attested that several root traits, including architecture, can be obtained from just a section of the root system (Fitter and Stickland, 1992). In this study, a developmental system was used to identify branching orders, thus the root axis was referred to as the main axis, and any lateral root emerging on the main axis was a first-order lateral root. Similarly, lateral roots emerging on first-order lateral roots were identified as second-order lateral roots, which in this study was the highest level examined (Fig. 1a). Root segments located 3 cm below the point of attachment to the shoot were sampled for analysis; these roots were the most developed part of the root system. The diameter of the axis and first- and second-order branches of this segment were measured with a magnifying lens (×7) equipped with a 0.1 mm scale. Ten measures per root order level were recorded to obtain a representative mean for the root segment. Each root segment was spread, avoiding root overlapping, on a transparent acetate sheet using dissecting needles and scanned at full size (300 dpi) for further

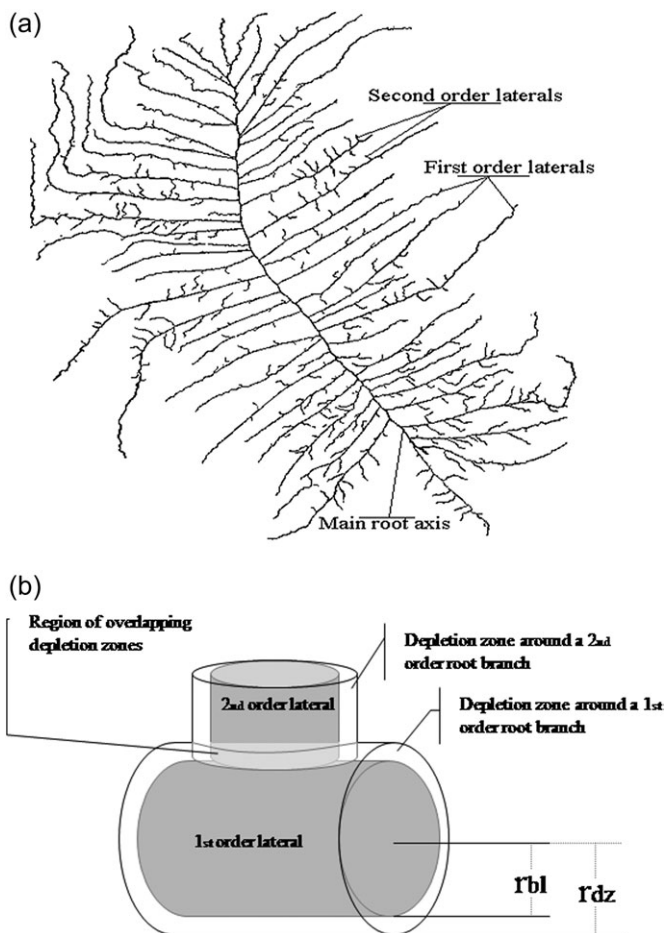


Fig. 1. Digitalized root segment (a) showing a developmental branching classification with the main root axis, and first- and second-order lateral root branches. The diagram in (b) depicts depletion zones for two connected root types and the region of overlapping depletion zones.

measurements. With a digitizing tablet (SummaSketch III, Summagraphics), each IBD along the main root axis and first-order branch of this segment was measured from a copy (Fig. 1a). The length of first-order branches was measured from the same 10 cm segment, whereas the length of each second-order branch was recorded from only a 5 cm central section within the 10 cm root segment. Values of root volume (cm³, assuming cylindrical roots), the ratio of root length to root mass [specific root length (SRL), m g⁻¹], and the ratio of root mass to root volume (M:V, g cm⁻³, an indicator of root tissue density) were calculated.

Model simulations

A model of nutrient diffusivity (Barber, 1984) was used to estimate two important root functional properties: exploitation potential [EP; volume of soil exploited by roots (Berntson, 1994)] and exploitation efficiency [EE; volume of soil exploited per unit volume of root (Fitter *et al.*, 1991)]. The relationship of root axis IBD to these two functional attributes of root systems, which were calculated from values of root length, root diameter for each root order (in the 10 cm root segment), and volume of the depletion zone, was examined. Root exploitation was adjusted by the growth (elongation) of the root, hence relative root elongation rates were calculated from additional data that included observations of root elongation for each nutrient treatment during three consecutive harvests (Arredondo and Johnson, 1999). Because values for root

elongation were for the entire root system, similar relative growth rates were assumed for the elongation of first- and second-order lateral roots. A simple model of nutrient diffusivity for nitrogen was used to calculate the radius of the depletion zone around each root (r_{dz}) using the following equation:

$$rdz = r_L + 2Dt \quad (1)$$

where r_L is the average radius for a particular root order, D is the diffusion coefficient of the ion in the soil, and t is time (Nye and Tinker, 1977; Fitter *et al.*, 1991; Berntson, 1994). A diffusion coefficient for nitrate in wet soil of $3.2 \times 10^{-9} \text{ cm}^2 \text{ s}^{-1}$ was used (Jackson and Caldwell, 1996). The cross-sectional area and volume (assuming cylindrical depletion zones) of soil exploited by the root were estimated for five consecutive days. The volume of the depletion zone (V_{dz}) was calculated separately by multiplying the cross-sectional area of the depletion zone by the summed length for each branch order level (V_{z1} for first-order roots and V_{z2} for second-order roots), assuming constant root diameter for each root order:

$$V_{dz} = V_{z1} + V_{z2} \quad (2)$$

Total depletion zone volume at any time was corrected for overlapping depletion zones between adjacent branch orders. This involved first calculating the area of a circular cross-section of the depletion zone for second-order branches. Then the difference between the radius of the depletion zone (r_{dz}) and the radius of the branch (r_{b1}) of first-order roots was used to calculate the volume of the hollow cylinder of overlapping depletion zones (Fig. 1b). This volume was multiplied by the number of second-order branches for each root sample. Finally, the volume of overlapping depletion zones was subtracted from V_{dz} . A similar procedure was used to correct for overlapping of first-order branches with the main root axis.

The output of the model was ‘volume of soil exploited’, which has been termed exploitation potential (EP_s) by Berntson (1994). The volume of soil exploited was divided by the root volume to calculate ‘the volume of soil exploited per unit volume of root’ (Berntson, 1994), which was equivalent to exploitation efficiency of the root system (EE_s ; the total volume of soil exploited per unit volume of root) (Fitter, 1987; Fitter *et al.*, 1991). Robinson *et al.* (1991) pointed out the limitations of models that simulate resource acquisition by roots because no distinction is generally made among the different functions of root orders. Thus, besides calculating the total values of EP_s and EE_s , the present study distinguished between the functional properties of first- and second-order root branches by estimating values for EP and EE independently for first- (EP_{1st} and EE_{1st}) and second- (EP_{2nd} and EE_{2nd}) order branches.

Statistics

Data were tested for normality using normal probability plots of residuals, stem-and-leaf diagrams, and the Shapiro–Wilk test (Zar, 1984). Non-normal data were logarithm transformed to correct for deviations from normality. Data for biomass and root traits were analysed by analysis of variance (ANOVA) as a factorial design with randomized complete blocks and four replications using mixed models (Proc MIXED) with blocks as a random factor (SAS Institute Inc., 2002–2003). The numbers of IBDs measured per root sample ranged from 25 to 65 for the axis and from 50 to 630 for first-order branches. Coefficients of variation (CVs) were examined for the IBD of the axis and first-order branches. Because CV values for IBDs were high (45–75%), Bartlett’s test for homogeneity of variances (Sokal and Rohlf, 1995) was used to examine equality of variances for each set of samples from the same treatment. Despite the high CV observed in IBDs, variances were generally similar, except in three cases involving crested wheatgrass. Allometric relationships between log-transformed

means of root morphological and functional traits were examined by fitting data points to a power function (Niklas, 1994):

$$Y_1 = \beta Y_2^\alpha \quad (3)$$

where α is the regression coefficient or scaling exponent. In some cases, the best fit was obtained with a simple linear function. Reduced-major-axis regression was used to assess the relationships among root traits. Confidence intervals for regression coefficients at $P \leq 0.05$ were used to compare scaling exponents (Sokal and Rohlf, 1995).

Results

Comparison of root traits among species and nutrient treatments

The three grass species exhibited large differences for all measured root characteristics ($P \leq 0.05$, Table 1); however, in general, no block effect was found. Overall, the annual cheatgrass exhibited an ~50% smaller IBD for the main root axis and first-order roots than the perennial crested wheatgrass and bluebunch wheatgrass (Table 2, $P \leq 0.01$), which had similar values of IBDs. For the length of first- and second-order lateral roots, the density of first-order lateral roots (average number of first-order laterals per unit length on main axis), the number of second-order lateral roots (total number of second-order lateral roots in the 5 cm root sample), total length of the root sample, and SRL, cheatgrass generally exhibited the highest values, bluebunch wheatgrass the lowest values, and crested wheatgrass intermediate values. However, crested wheatgrass produced nearly twice as much root biomass (whole root system) compared with cheatgrass and bluebunch wheatgrass. The M:V ratio (an indicator of tissue density) was >2-fold greater for bluebunch wheatgrass compared with cheatgrass and crested wheatgrass.

Regarding the effect of nutrient treatments on root growth, the length of first-order IBDs was shorter under fertile soil nutrient conditions (UH and NUH) and longer under the poorest soil nutrient condition (UL) (Table 3). Lengths of second-order laterals were greater in the UH treatment compared with those from the NUL and UL treatments. The number of second-order branches, root dry weight, and total root length were higher in UH and NUH than in NUL and UL treatments.

IBD relationships between the root axis and first-order root branches

Regression analysis for individual species showed a significant negative relationship between log IBD of the main axis and the log of first-order roots in bluebunch wheatgrass ($r^2=0.95$, $n=4$, $P=0.047$), but not for cheatgrass and crested wheatgrass ($r^2=0.48$, $n=4$, $P=0.30$, and $r^2=0.01$, $n=4$, $P=0.86$, respectively). For bluebunch wheatgrass, a decrease in soil nutrient availability (UH → NUH → NUL → UL) resulted in a reduction of main axis IBD with an opposite increase in first-order IBD (Fig. 2).

Table 1. ANOVA and associated sum of squares for interbranch distances in the main root axis (MA-IBD) and in first-order lateral roots (FO-IBD), length of first-order (Lgth. 1st) and second-order (Lgth. 2nd) lateral roots, density of first-order lateral roots (Dens. 1st), number of second-order lateral roots in all first-order branches in the root sample (Num. 2nd), root dry weight (DW root), total length of the root sample (Tot. lgth.), specific root length (SRL), and the ratio of root mass to root volume (M:V ratio) with species (S) and nutrient treatment (N) as main factors

Source of variation	df	MA-IBD	FO-IBD	Lgth. 1st	Lgth. 2nd	Dens. 1st	Num. 2nd	DW root	Tot. lgth.	SRL	M:V ratio
Species (S)	2	0.7540*	0.3057**	0.7518**	1.8766*	0.8271**	3.0531**	0.5007**	2.4554**	2.2049**	1.1614**
Nutrient (N)	3	0.0037	0.2988**	0.2238	5.8055**	0.0079	4.1821**	1.8827**	3.3505**	0.2467	0.2022
S×N	6	0.0701	0.1528	0.3991	0.9901	0.0508	0.8865	0.4435**	0.2964	0.1319	0.3025

* and ** indicate significant differences at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 2. Means of interbranch distances in the main root axis (MA-IBD) and in first-order lateral roots (FO-IBD), length of first-order (Lgth. 1st) and second-order lateral roots (Lgth. 2nd), density of first-order lateral roots (Dens. 1st), number of second-order lateral roots in all first-order branches in the root sample (Num. 2nd), root dry weight (DW root), total length of the root sample (Tot. lgth.), specific root length (SRL), and the ratio of root mass to root volume (M:V ratio) for three range grasses

Species	MA-IBD (cm)	FO-IBD (cm)	Lgth. 1st (cm)	Lgth. 2nd (cm)	Dens. 1st (per 10 cm)	Num. 2nd (per 5 cm)	DW root (g)	Tot. lgth. (cm)	SRL (m g ⁻¹)	M:V ratio (g cm ⁻³)
Cheatgrass	0.154 a	0.398 a	105.4 a	43.2 a	65 a	140.4 a	0.0077 b	191.3 a	631 a	0.33 b
Crested wheatgrass	0.285 b	0.536 b	69.3 b	26.8 a,b	34 b	92.6 a	0.0123 a	135.4 a	279 b	0.26 b
Bluebunch wheatgrass	0.299 b	0.640 b	50.0 b	13.2 b	32 b	35.1 b	0.0073 b	55.6 b	194 c	0.60 a

Values within a column followed by different letters are significantly different at $P \leq 0.05$.

Table 3. Means of interbranch distances for the main axis (MA-IBD) and first-order lateral roots (FO-IBD), length of first-order (Lgth. 1st) and second-order lateral roots (Lgth. 2nd), density of first-order lateral roots (Dens. 1st), number of second-order lateral in all first-order branches in root sample (Num. 2nd), root dry weight (DW root), total length of the root sample (Tot. lgth.), specific root length (SRL), and the ratio of root mass to root volume (M:V ratio) for four nutrient treatments: uniform high (UH), non-uniform high (NUH), non-uniform low (NUL), and uniform low (UL)

Nutrient treatment	MA-IBD (cm)	FO-IBD (cm)	Lgth. 1st (cm)	Lgth. 2nd (cm)	Dens. 1st (per 10 cm)	Num. 2nd (per 5 cm)	DW root (g)	Tot. lgth. (cm)	SRL (m g ⁻¹)	M:V ratio (g cm ⁻³)
UH	0.244 a	0.387 c	82.5 a	78.4 a	40 a	159.6 a	0.0130 a	208 a	407 a	0.30 a
NUH	0.238 a	0.481 bc	86.6 a	38.6 a,b	41 a	142.4 a	0.0149 a	206 a	351 a	0.34 a
NUL	0.231 a	0.590 ab	62.3 a	15.5 b,c	41 a	44.7 b	0.0057 b	59 b	267 a	0.43 a
UL	0.232 a	0.640 a	58.9 a	8.14 c	44 a	34.3 b	0.0056 b	64 b	289 a	0.43 a

Values within a column followed by different letters are significantly different at $P \leq 0.05$.

Relationships between main axis IBD and root morphological traits

Examination of allometric relationships in bluebunch wheatgrass showed a positive association between the main axis IBD and the length and number of second-order roots and SRL, whereas the main axis IBD was negatively associated with the number of first-order roots and the M:V ratio (Fig. 3a–e). In this case, the largest root axis IBD, greatest length and number of second-order roots, and highest SRL occurred under high-nutrient conditions (UH and NUH), which were also associated with the lowest number of first-order roots and lowest M:V ratio (decreased root tissue density). For cheatgrass, the root axis IBD was negatively associated with the number of first- and second-order roots

and total length of second-order roots (Fig. 4a–c). For cheatgrass, the low-nutrient treatments (NUL and UL) resulted in the largest distance between neighbouring branches along the main axis as well as the smallest number and length of first- and second-order roots. Although the main axis IBD was positively related to the M:V ratio in crested wheatgrass (on a log scale), no other consistent relationships were observed with nutrient availability.

Model output for root functional properties

Five-day model simulations showed distinctive patterns of EP and EE for the three range grasses and nutrient conditions (Figs 5, 6). For example, model simulations showed similar magnitudes of EP for both cheatgrass and

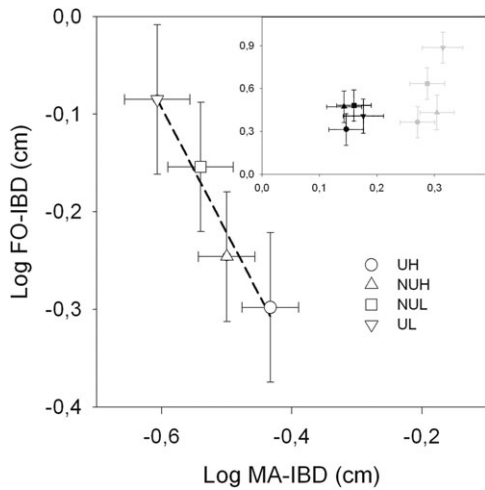


Fig. 2. Log-log plots (base 10) between the average interbranch distance of the main root axis (MA-IBD) and the average interbranch distance of first-order root branches (FO-IBD) for bluebunch wheatgrass. Vertical and horizontal error bars correspond to ± 1 SE. The inset in the figure shows a similar relationship for cheatgrass (black symbols) and crested wheatgrass (grey symbols). Statistics for regression are: $r^2=0.95$, $P=0.047$; $r^2=0.48$, $P=0.30$; and $r^2=0.01$, $P=0.86$, for bluebunch wheatgrass, cheatgrass, and crested wheatgrass, respectively.

crested wheatgrass, whereas EP values for bluebunch wheatgrass were considerably lower. Considering nutrient treatments, cheatgrass exhibited similar EP values when grown in the UH and NUH treatments (Fig. 5a), which for plants in the NUH treatment was due to a high EP_{1st} (Fig. 5b) and for plants in the UH treatment was due to a high EP_{2nd} (Fig. 5c). The EP for cheatgrass was always lowest in the UL treatment (Fig. 5a–c). Crested wheatgrass exhibited the most consistent EP response patterns with respect to nutrient treatments, with the highest EP_s , EP_{1st} , and EP_{2nd} in the UH treatment followed by the NUH, NUL, and UL treatments (Fig. 5d–f). Bluebunch wheatgrass displayed the least consistent EP response pattern (Fig. 5g–i), with the lowest values of EP_s , EP_{1st} , and EP_{2nd} occurring in the NUL treatment. Although EP_s and EP_{2nd} for bluebunch wheatgrass were highest in the UH treatment, EP_{1st} was greatest in the UL treatment.

Model simulations showed considerably greater values of EE for cheatgrass compared with crested wheatgrass and bluebunch wheatgrass (Fig. 6). The highest values of EE_s and EE_{1st} in cheatgrass were observed in the UH treatment followed by the UL, NUL, and NUH treatments (Fig. 6a, b). For cheatgrass, a slightly higher EE_{2nd} was detected in the NUH compared with the UH treatment (Fig. 6c). For crested wheatgrass, values of EE_s , EE_{1st} , and EE_{2nd} were highest in the NUH treatment (Fig. 6d–f). Values of EE_s , EE_{1st} , and EE_{2nd} for bluebunch wheatgrass did not differ among nutrient treatments (Fig. 6g–i).

Main axis IBD relationships to EP and EE

For cheatgrass, decreased nutrient availability was associated with longer main axis IBD and accompanying reductions in

EP_s and EP_{1st} (Fig. 7a, b, $r^2=0.98$ and $r^2=0.91$, respectively, $P \leq 0.05$, $n=4$); however, no relationship was found between axis IBD and EE for any nutrient treatment (data not shown). Although the main axis IBD and EP_{2nd} for bluebunch wheatgrass increased in the NUH and UH treatments as compared with the UL and NUL treatments (Fig. 8a, $r^2=0.95$, $P \leq 0.05$, $n=4$), EE_{1st} decreased in the UH compared with the UL treatment (Fig. 8b, $r^2=0.93$, $P \leq 0.05$, $n=4$). The relationship between main axis IBD and EP_{2nd} became weaker through time, while the relationship between IBD and EE_{1st} remained unchanged during the 5 d simulation period (data not shown). No significant relationships were observed between main axis IBD, EE, and EP for crested wheatgrass.

Discussion

Concerning the morphological trade-offs in root IBD, three scenarios are possible regarding how IBD on the main root relates to IBD on the next branch order (e.g. main axis versus first-order laterals): no relationship, positive, or negative relationship. Functional interpretations of these responses are similar to those of other root foraging responses. For instance, it has been suggested that a large number of small, fine roots are needed to acquire immobile soil resources rapidly (Scott Russell and Clarkson, 1976; Caldwell and Richards, 1986), improve competitive ability (Hodge *et al.*, 1999; Robinson *et al.*, 1999), or enhance the exploitation potential of the root system (Berntson, 1994). In the present study, for plants exposed to increased soil fertility, root foraging responses may include roots with either: (i) increased main axis IBD combined with a decreased first-order IBD (negative relationship); or (ii) decreased main axis IBD combined with decreased first-order IBD (positive relationship). In both cases, a decrease of IBD in first-order roots translates into increases in root branch density and subsequently the length of second-order root branches. In contrast, for plants exposed to low-nutrient soil conditions, predicted responses could include either: (i) increased IBD in the main root axis together with increased IBD in first-order branches (positive relationship); or (ii) decreased IBD in the main root axis together with increased IBD in first-order branches (negative relationship). Thus, increased IBD in first-order branches may occur with low branching density and root length of second-order branches, delaying the overlap between depletion zones of neighbouring branches and at the same time improving root exploitation efficiency (Fitter *et al.*, 1991).

Allometry between main root axis IBD and first-order IBD

As mentioned earlier, the study of the proportional relationships between size, shape, and function of part or the whole organism is traditionally known as allometry (Gould, 1966; Reiss, 1989) or scaling analysis (Niklas, 1994). The results of the present study support the hypothesis that species which rely more on root architectural plasticity rather than fast root growth develop allometric relationships between

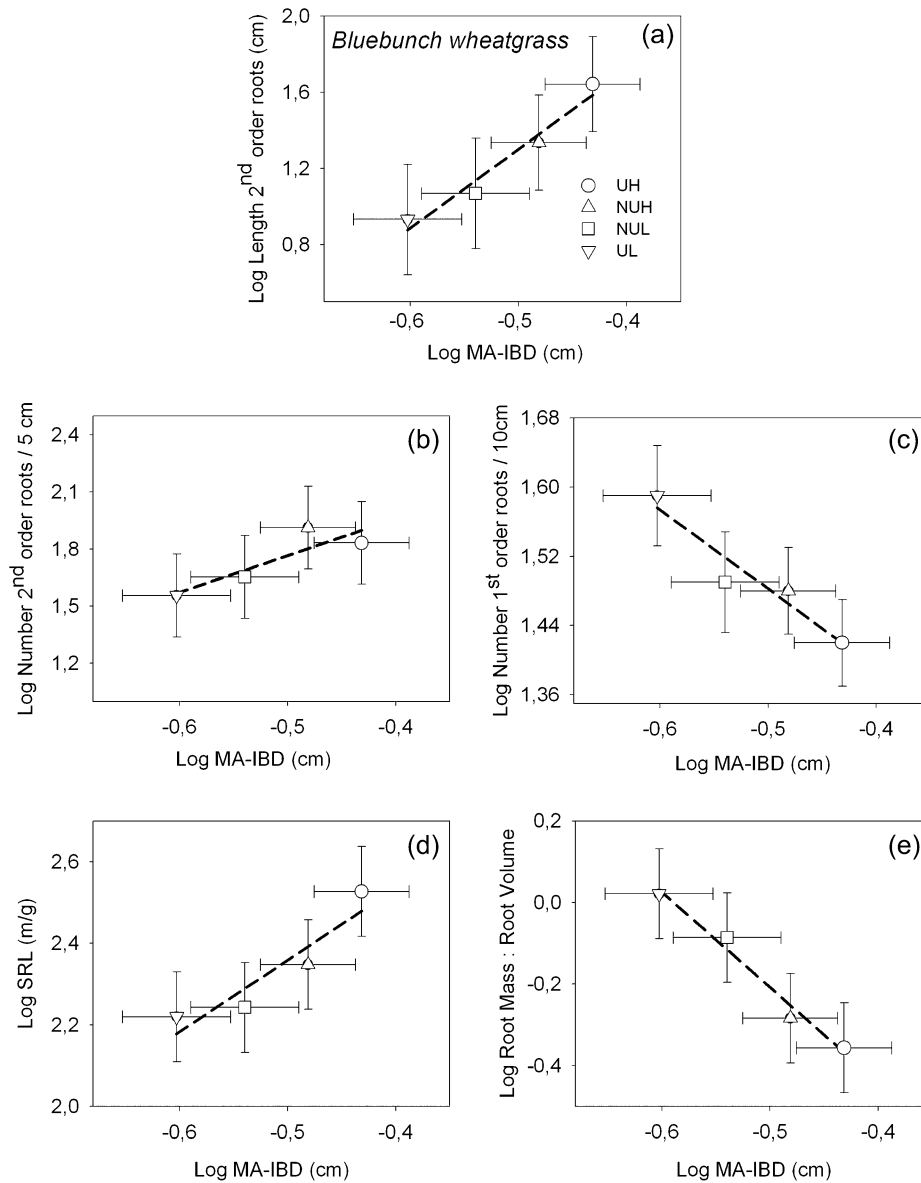


Fig. 3. Log–log plots (base 10) between the average interbranch distance of the main root axis (MA-IBD) and the length of second-order root branches (a), number of second-order root branches (b), number of first-order root branches (c), specific root length (SRL) (d), and the ratio between root mass and root volume (e) for bluebunch wheatgrass. Vertical and horizontal error bars correspond to ± 1 SE. Statistics for regression are: $r^2=0.93$, $P=0.022$; $r^2=0.96$, $P=0.012$; $r^2=0.78$, $P=0.06$; $r^2=0.79$, $P=0.06$; and $r^2=0.96$, $P=0.011$, respectively.

IBDs of different root orders. Thus, bluebunch wheatgrass, a species that responds to soil nutrient enrichment through architectural plasticity (Arredondo and Johnson, 1999), was the only species where the IBD of both the root axis and first-order roots followed an allometric relationship (Fig. 2). In contrast, both cheatgrass and crested wheatgrass (previously reported as displaying root proliferation) did not exhibit allometric relationships for these traits (Fig. 2, inset). For bluebunch wheatgrass, growth under a gradient of contrasting soil nutrient conditions (UH > NUH > NUL > UL) resulted in changes from a long IBD on the main root axis and short IBD for first-order lateral roots to a short IBD on the main root axis and long IBD for first-order lateral roots (negative relationship, Fig. 2).

The observed changes in the allometric IBD relationship suggest dynamic adjustments in root growth and root foraging strategies with changes in nutrient availability. As soils became less fertile, the results showed an increase in first-order IBD that was associated with a decline in the number and length of second-order roots (Fig. 3a, b), which was a result of increases in dry matter allocation to roots, as indicated by lower SRL and an increased M:V ratio (Fig. 3d). This root plasticity apparently helped to change the root system from a morphology that is more efficient at acquiring resources (i.e. exploitation potential in second-order roots, Fig. 8a) under high-nutrient conditions (UH and NUH) to a root morphology more apt to improve the efficiency of nutrient acquisition, such as when exposed to low-nutrient conditions (NUL and UL)

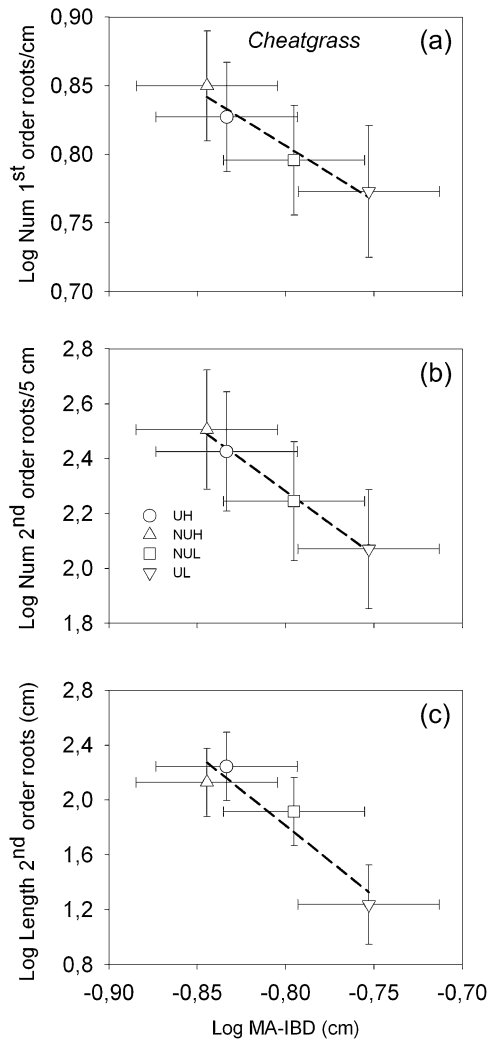


Fig. 4. Log-log plots (base 10) between the average interbranch distance of the main root axis (MA-IBD) and the number of first-order root branches (a), number of second-order root branches (b), and length of second-order root branches (c) for cheatgrass. Vertical and horizontal error bars correspond to ± 1 SE. Statistics for regression are: $r^2=0.93$, $P=0.022$; $r^2=0.99$, $P=0.002$; $r^2=0.85$, $P=0.05$, respectively.

(i.e. exploitation efficiency in first-order roots, Fig. 8b). A previous assessment of root architectural responses to soil nutrients in bluebunch wheatgrass (Arredondo and Johnson, 1999) showed similar patterns in which high soil nutrient availability favoured the formation of shorter internal–internal (II) links, which are equivalent to the IBDs reported here (II includes IBDs in all root orders). Similarly, previous theoretical (Fitter *et al.*, 1991; Berntson, 1994) and experimental work (Fitter and Stickland, 1991; Taub and Goldberg, 1996) suggested that traits including root enlargement, proliferation (i.e. lateral root initiation), and increases in root branching (i.e. dichotomous topology) would favour nutrient acquisition under enriched soil conditions. Thus, the allometry in IBD observed in this study agrees with the generally recognized relationships of root foraging, but, in addition, the results showed complementary functional roles of different root branch order levels in root foraging mechanisms.

If changes at these levels are coordinated as proposed in the first hypothesis, this allometric relationship should result in either negative or positive associations between the main axis IBD and several morphological and functional root traits. In bluebunch wheatgrass, the present study found a negative relationship between IBD on the main root axis and the number of first-order lateral roots (Fig. 3c). This negative relationship is consistent with the concept of root system coordination proposed by Hodge (2009), where plants use environmental cues to promote or curtail root growth. Allometric relationships for IBD of different root orders was not observed in crested wheatgrass and cheatgrass, both of which have rapid root growth responses, which is in agreement with the hypothesis. A possibility exists that in species that display inherently rapid root growth and lateral root initiation following a pulse of soil nutrients, the correlative mechanisms that control distance of branch initiation (IBD) might limit rapid root responses. Correlative mechanisms involving IBD allometric relationships are aligned with the concept of ‘precision foraging’ (Campbell *et al.*, 1991) by which slow-growing plants exhibit great accuracy in strategically locating their roots in enriched soil sites.

Allometry between main root axis IBD and other root traits

For bluebunch wheatgrass and cheatgrass, the average IBD observed in the four soil nutrient treatments was related to root morphology and functional properties of roots, which supports the second hypothesis put forward. Although cheatgrass and crested wheatgrass did not exhibit allometric relationships between IBDs, the results showed that main axis IBD was associated with several other morphological and functional root traits, at least for cheatgrass. The observed relationships of main axis IBD to several morphological and functional root properties at different root orders suggest that branch order exhibits distinctive functional roles within the root system, which is supported by results of other studies (Eissenstat and Yanai, 1994; Eshel and Waisel, 1996). In particular, the work of Guo *et al.* (2008) and Valenzuela-Estrada *et al.* (2008) showed that root branch order was closely related to anatomical root characteristics, which in turn were related to functional roles of various root orders.

In this study, it is perhaps not surprising that EP in bluebunch wheatgrass changed for second-order root branches, while EE changed for first-order branches. Nutrient uptake is known to occur in the first 2 cm of root tips (Scott Russell and Clarkson, 1976; Eshel and Waisel, 1996). In the grasses studied, second-order branches were the most distal roots that included root tips measuring <2 cm in length and, therefore, actively involved in resource acquisition. Environmental effects that produce changes in the most distal roots (e.g. second-order roots) may then in turn affect the amount of soil exploited (EP). A study examining root function of different root orders in 23 Chinese temperate tree species (Guo *et al.*, 2008) showed

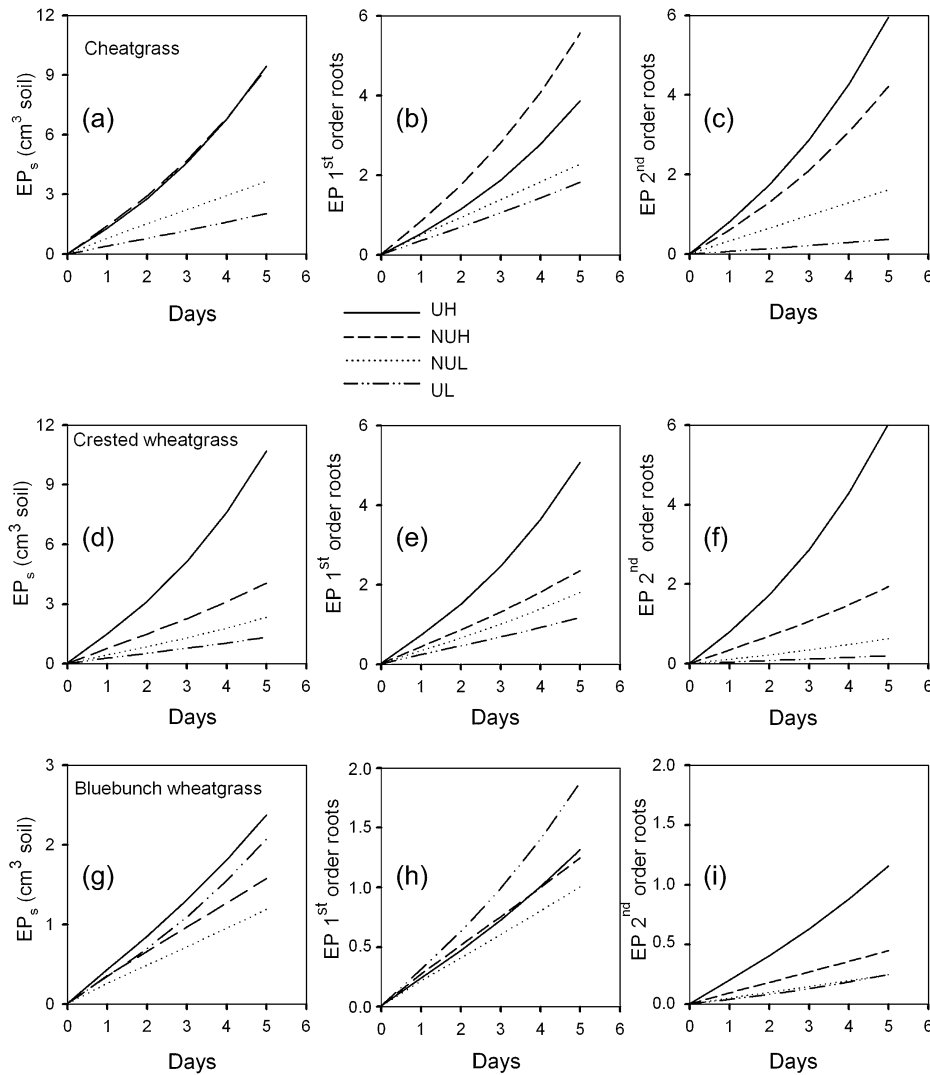


Fig. 5. Depiction of 5th d of simulated exploitation potentials for the total root sample (EP_s), first-order root branches (EP_{1st}), and second-order root branches (EP_{2nd}) for cheatgrass (a, b, c), crested wheatgrass (d, e, f), and bluebunch wheatgrass (g, h, i).

that 75% of most distal roots were involved in uptake. Root uptake capacity of this root type, however, declines rapidly with age, as demonstrated by Volder *et al.* (2005). The ageing effect on different root orders was not incorporated into the simulation of EP in the roots in the present study. In contrast, first-order root branches appear to have a role in the efficient distribution of second-order roots, which could improve EE and complement EP of the root system. The importance of EE_{1st} probably increased as local nutrient availability became more limiting with concurrent decreases in EP_{2nd}, which is similar to the results of Berntson (1994) concerning the trade-offs between EE and EP for whole root systems.

The similar allometric coefficients for the relationships of main root axis IBD and morphology (e.g. length of second-order branches) with main root axis IBD and functional attributes (e.g. EP_{2nd}) of the same root order observed in bluebunch wheatgrass and cheatgrass suggests that root branching design follows developmental rules governed by soil nutrient availability, plant nutrient

demand and acquisition, interbranch competition, source-sink equilibrium, and carbon distribution (Hodge, 2009). Other studies have reported similar allometric relationships between mean II link length and relative growth rate, SRL, and M:V root ratio (Arredondo and Johnson, 1999), as well as root biomass of neighbouring plants (Janeček *et al.*, 2007). The similar allometric coefficients between root length and EP may have arisen as an artefact with the use of root length to calculate EP. However, it is surprising that similar allometric relationships were not observed in crested wheatgrass and that these relationships differed between bluebunch wheatgrass and cheatgrass (data not shown). This suggests that the allometric relationships between the main root axis IBD and functional root properties probably occurred as a result of changes in multiple traits. These other traits might include root diameter, which is a trait used in calculating EP and EE and has a large impact on root length and root tissue density (M:V ratio), that is important in determining costs of root responses (Eissenstat, 1992).

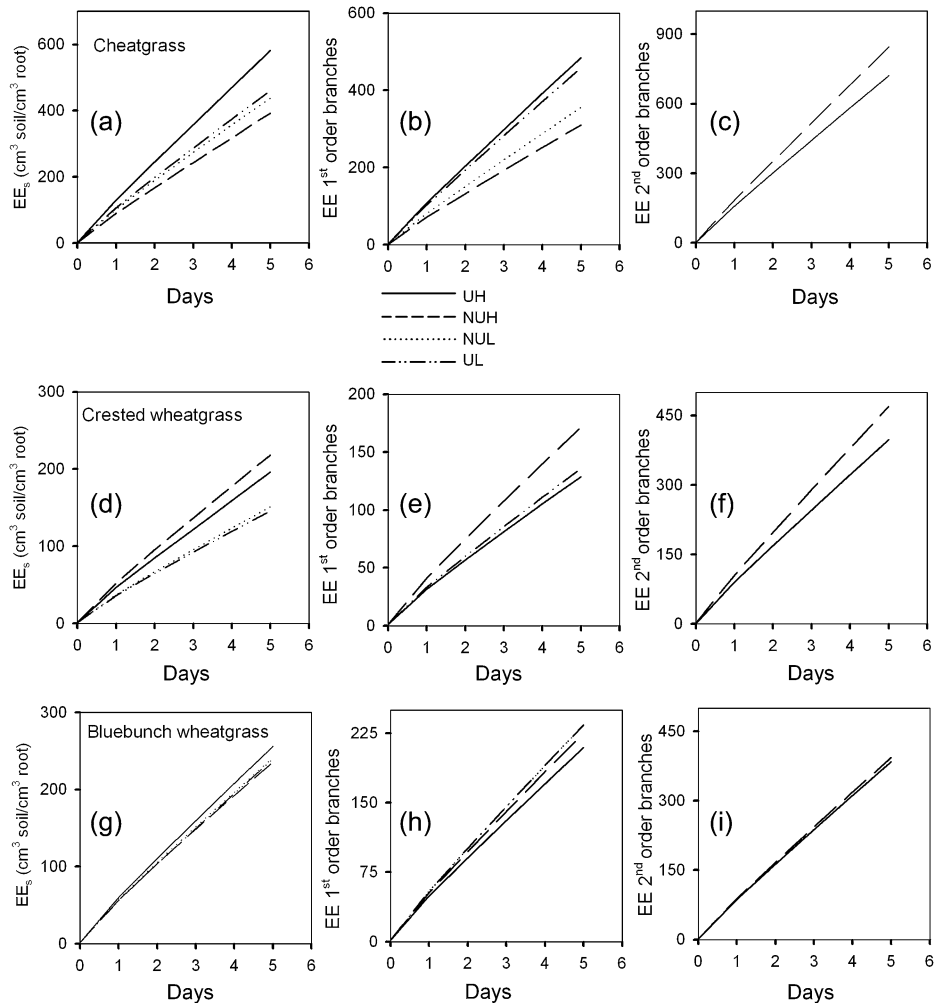


Fig. 6. Depiction of 5^d of simulated exploitation efficiencies (EE) for the total root sample (EE_s), first-order root branches (EE_{1st}), and second-order root branches (EE_{2nd}) for cheatgrass (a, b, c), crested wheatgrass (d, e, f), and bluebunch wheatgrass (g, h, i).

In this study, important differences were observed in root diameter and the M:V ratio among species and nutrient conditions. In general, the responses of the M:V ratio for all three grasses examined here (data not presented) agreed with previous studies that showed a decrease in the M:V ratio under fertile soil conditions and an increase in the M:V ratio under low fertility conditions (Robinson *et al.*, 1999). However, allometric relationships were only observed in the present study between the main root axis IBD and the M:V ratio in bluebunch wheatgrass. Thus, besides exhibiting a large root architectural plasticity (Arredondo and Johnson, 1999), bluebunch wheatgrass also displayed large biomass allocation following strict allometric control. In their study of morphological and functional traits among various root orders in *Vaccinium corymbosum*, Valenzuela-Estrada *et al.* (2008) found that variation in the M:V ratio was coordinated with changes in root length, root biomass, SRL, and C:N ratio. The present results also indicated that specific root responses, such as those observed for second-order root branches, were part of integrated adjustments that occurred in the whole root system. For example, in bluebunch wheatgrass, increases in the length and number of second-order root branches under nutrient-enriched

conditions (UH and NUH, Fig. 3a, b) apparently resulted from decreases in the number of first-order laterals and the M:V ratio (Fig. 3e) as well as increases in SRL (Fig. 3d). Crested wheatgrass and cheatgrass did not exhibit this integrated response to soil nutrient availability as observed in bluebunch wheatgrass, perhaps indicating a limitation to express correlative mechanisms to soil nutrient pulses (see above). Still, these two species with rapid root growth response to soil nutrient enrichment showed plasticity in individual traits such as SRL, root length, root diameter, etc.

Adaptive and ecological consequences of IBD allometry

The concurrent changes observed between the main axis IBD and several other morphological traits support the argument that branch spacing along the main root axis may have an adaptive role in regulating various ecological and functional aspects of branching structures in roots such as: (i) delaying interbranch competition; (ii) establishing the functional role of roots; (iii) regulating biomass distribution in roots; and perhaps (iv) affecting the life span of roots.

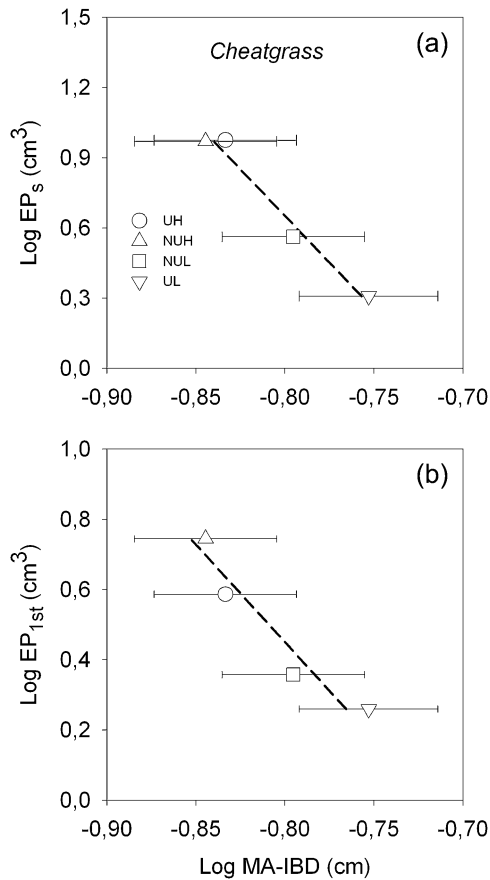


Fig. 7. Log–log plots (base 10) between the interbranch distance of the main root axis (MA-IBD) and the exploitation potential for the total root sample (EP_s) (a) and for first-order root branches (b) observed after 5^d for cheatgrass. Horizontal error bars correspond to ± 1 SE. Statistics for regression are: $r^2=0.96$, $P=0.012$ and $r^2=0.96$, $P=0.01$, respectively.

Regulation of interbranch competition can be inferred from the negative relationship between main axis IBD and first-order IBD for bluebunch wheatgrass (Fig. 2). As explained before, delaying of interbranch competition may occur by spacing second-order branches at a greater distance, similar to those observed in nutrient-poor (UL and NUL) conditions. With this greater spacing, overlapping of depletion zones will be postponed for second-order branches, the physiologically most active roots (Scott Russell and Clarkson, 1976). These correlative changes in IBD were not observed in either cheatgrass or crested wheatgrass. For a short-lived annual grass such as cheatgrass, rapid acquisition of soil resources by fine roots that turn over rapidly may counter the effects of overlapping depletion zones.

Regulation of root functional attributes by IBD in the main root axis was derived from the relationship between the density and length of first- and second-order branches (Fig. 3). For bluebunch wheatgrass, changes in the IBD of the main root axis along the nutrient gradient were associated with changes in traits of both first-order branches (EE_{1st}) and second-order branches (EP_{2nd}). However, for cheatgrass, changes in the IBD of the main root

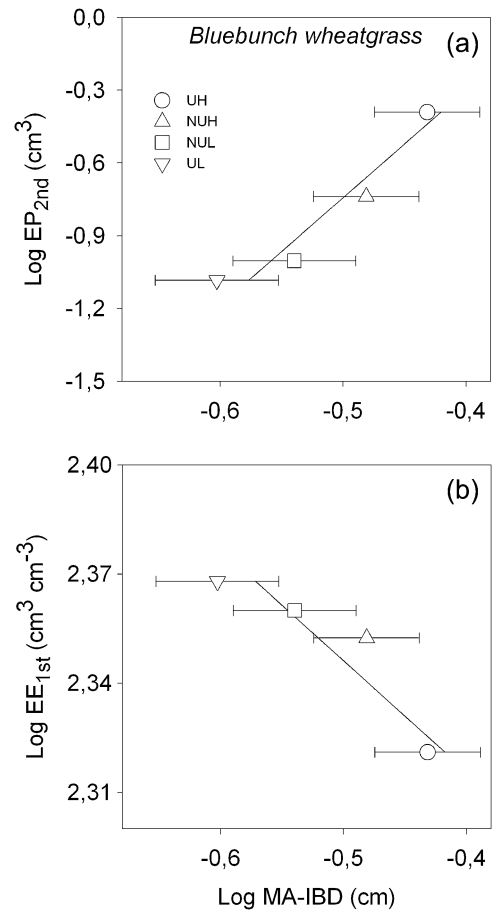


Fig. 8. Log–log plots (base 10) between the average interbranch distance for the main root axis (MA-IBD) and the exploitation potential of second-order root branches (EP_{2nd}) (a) and the exploitation efficiency of first-order root branches (EE_{1st}) (b) observed after 5^d for bluebunch wheatgrass. Horizontal error bars correspond to ± 1 SE. Statistics for regression are: $r^2=0.89$, $P=0.037$ and $r^2=0.94$, $P=0.02$, respectively.

axis only coincided with changes in second-order branches (EP_{2nd}; Fig. 4). For bluebunch wheatgrass, these relationships suggest that each branch order may have different functional roles in the root system, as suggested previously by Guo *et al.* (2008) and Valenzuela-Estrada *et al.* (2008). The greatest nutrient uptake capacity occurs in second-order roots (Scott Russell and Clarkson, 1976; Pregitzer, 2002; Guo *et al.*, 2008). In contrast, first-order branches may play a role in efficiently distributing acquired nutrients and improving EE, which would be particularly important as soil conditions become less fertile. Cheatgrass may only emphasize EP, which is a typical growth strategy exhibited by a ruderal, highly competitive, and fast-growing species (Grime, 1979).

Main axis IBD characteristics may also play an important role in the way biomass is distributed in roots. For example, increases in the number and length of second-order branches in enriched nutrient conditions (UH and NUH; Fig. 3) coincided with decreases in root tissue density (M:V ratio) and increases in SRL (length produced per unit biomass). Plasticity in biomass allocation has an important

role in root foraging of soils because changes in the M:V ratio and SRL have a large impact on carbon costs (Eissenstat, 1991). Species with the capability to project roots into enriched sites using less carbon (e.g. low tissue density or low M:V ratio) will have an advantage in acquiring soil resources (Robinson, 1999). Arredondo and Johnson (1999) showed that the same species as in the present study differed in root tissue density characteristics and that this trait varied with soil nutrient level. In the long term, changes in root tissue density may affect the life span of roots. The life span of roots with a high M:V ratio can be increased because organs with higher bulk tissue density typically have higher longevity (Ryzer, 1996). For herbaceous plants, root tissue composition (e.g. M:V ratio) could be as important as other root traits such as root diameter in terms of root life span.

Conclusions

The present results suggest that correlative mechanisms controlling root initiation and root emergence (Malamy, 2005) may produce branching patterns that exhibit regularity and that respond to nutrient gradients, such as those observed for bluebunch wheatgrass and cheatgrass. Changes in branching patterns may initiate at the IBD in the main root axis and be associated with changes in IBDs at higher root orders, root length, and number of roots, which may contribute to the optimization of root biomass distribution and root functional properties. Characteristics of the main axis IBD in some species may reflect growth rules for higher order root branches, even in cases where IBD was not related between sequential branch orders, such as was observed here in cheatgrass. Previous studies with other species using root architectural approaches showed similar results (Fitter *et al.*, 1991; Nielsen *et al.*, 1994; Berntson, 1994). The approach used here is similar to that of Fitter *et al.* (1991), which is based on a link classification system where changes in root link length can be related to changes in functional properties of the whole root system. However, the present approach differs because the root system can be evaluated in terms of how changes in root branching influence other morphological and allocation traits and functional properties of particular root orders. Elucidation of these branching patterns under diverse environmental conditions and in other plant species will help facilitate more accurate simulation modelling of root growth.

Acknowledgements

The authors thank K. Connors for his excellent technical assistance, and E. Huber-Sannwald for her comments on an earlier version of the manuscript. Data in this paper were collected while JTA was a graduate research assistant at Utah State University with DAJ as Major Professor.

References

- Arredondo JT, Johnson DA.** 1999. Root architecture and biomass allocation of three range grasses in response to non-uniform supply of nutrients and shoot defoliation. *New Phytologist* **143**, 373–385.
- Barber SA.** 1984. *Soil nutrient bioavailability*. New York: Wiley-Interscience.
- Berntson GM.** 1994. Modelling root architecture: are there tradeoffs between efficiency and potential of resource acquisition? *New Phytologist* **127**, 483–493.
- Caldwell MM, Richards JH.** 1986. Competing root systems: morphology and models of absorption. In: Givnish TJ, ed. *On the economy of plant form and function*. New York: Cambridge University Press, 251–273.
- Campbell BD, Grime JP, Mackey JML.** 1991. A trade-off between scale and precision in resource foraging. *Oecologia* **87**, 532–538.
- Charlton WA.** 1996. Lateral root initiation. In: Waisel Y, Eshel A, Kafkafi U, eds. *Plant roots: the hidden half*. New York: Marcel Dekker, 149–174.
- Crick JC, Grime JP.** 1987. Morphological plasticity and mineral nutrient capture in two herbaceous species of contrasted ecology. *New Phytologist* **107**, 403–414.
- Dubrovsky JG, Doerner PW, Colon-Carmona A, Rost TL.** 2000. Pericycle cell proliferation and lateral root initiation in *Arabidopsis*. *Plant Physiology* **124**, 1648–1657.
- Eissenstat DM.** 1991. On the relationship between specific root length and the rate of root proliferation: a field study using citrus rootstocks. *New Phytologist* **118**, 63–68.
- Eissenstat DM.** 1992. Costs and benefits of constructing roots of small diameter. *Journal of Plant Nutrition* **15**, 763–782.
- Eissenstat DM, Caldwell MM.** 1988. Seasonal timing of root growth in favorable microsites. *Ecology* **69**, 870–873.
- Eissenstat DM, Yanai RD.** 1994. The ecology of root life span. *Advances in Ecological Research* **27**, 2–60.
- Ennos AR, Fitter AH.** 1991. The mechanics of anchorage in wheat *Triticum aestivum* L. The anchorage of wheat seedlings. *Journal of Experimental Botany* **42**, 1601–1606.
- Ernel FF, Vizoso S, Charpentier JP, Jay-Allemand C, Cateson AM, Couee I.** 2000. Mechanisms of primordium formation during adventitious root development from walnut cotyledon explants. *Planta* **211**, 563–574.
- Eshel A, Waisel Y.** 1996. Multiform and multifunction of various constituents of one root system. In: Waisel Y, Eshel A, Kafkafi U, eds. *Plant roots: the hidden half*. New York: Marcel Dekker, 175–192.
- Fitter AH.** 1987. A comparative approach to the comparative ecology of plant root systems. *New Phytologist* **106**(suppl), 61–77.
- Fitter AH, Stickland TR.** 1991. Architectural analysis of plant root systems. 2. Influence of nutrient supply on architecture in contrasting plant species. *New Phytologist* **118**, 383–389.
- Fitter AH, Stickland TR.** 1992. Architectural analysis of plant root systems. 3. Studies on plants under field conditions. *New Phytologist* **121**, 243–248.

- Fitter AH, Stickland TR, Harvey ML, Wilson GW.** 1991. Architectural analysis of plant root systems. 1. Architectural correlates of exploitation efficiency. *New Phytologist* **118**, 375–382.
- Forde BG, Walch-Liu P.** 2009. Nitrate and glutamate as environmental cues for behavioral responses in plant roots. *Plant, Cell and Environment* **32**, 682–693.
- Gould SJ.** 1966. Allometry and size in ontogeny and phylogeny. *Biology Review* **41**, 587–640.
- Grime JP.** 1979. *Plant strategies and vegetation processes*. New York: Wiley.
- Guo D, Xia M, Wei X, Chang W, Liu Y, Wang Z.** 2008. Anatomical traits associated with absorption and mycorrhizal colonization are linked to root branch order in twenty-three temperate tree species. *New Phytologist* **180**, 673–683.
- Hewitt EJ.** 1966. *Sand and water culture methods used in the study of plant nutrition*, 2nd edn Technical Communication 22. East Malling, Kent: Commonwealth Agricultural Bureaux.
- Hodge A.** 2009. Root decisions. *Plant, Cell and Environment* **32**, 628–640.
- Hodge A, Robinson D, Griffiths BS, Fitter AH.** 1999. Why plants bother: root proliferation results in increased nitrogen capture from an organic patch when two grasses compete. *Plant, Cell and Environment* **22**, 811–820.
- Honda H, Fisher JB.** 1978. Tree branch angle: maximizing effective leaf area. *Science* **199**, 888–889.
- Jackson RB, Caldwell MM.** 1989. The timing and degree of root proliferation in fertile-soil microsites for three cold-desert perennials. *Oecologia* **81**, 149–153.
- Jackson RB, Caldwell MM.** 1996. Integrating resource heterogeneity and plant plasticity: modelling nitrate and phosphate uptake in a patchy soil environment. *Journal of Ecology* **84**, 891–903.
- Janeček Š, Janečková P, Leps J.** 2007. Effect of competition and soil quality on root topology of perennial grass *Molinia caerulea*. *Preslia* **79**, 23–32.
- Küppers M.** 1989. Ecological significance of above-ground architectural patterns in woody plants: a question of cost–benefit relationships. *Trends in Ecology and Evolution* **4**, 375–379.
- LeFèvre J.** 1983. Teleonomical optimization of a fractal model of the pulmonary arterial bed. *Journal of Theoretical Biology* **102**, 225–248.
- Malamy JE.** 2005. Intrinsic and environmental response pathways that regulate root system architecture. *Plant, Cell and Environment* **28**, 67–77.
- Mallory TE, Chang SH, Cutter EG, Gifford EM.** 1970. Sequence and pattern of lateral root formation in five selected species. *American Journal of Botany* **57**, 800–809.
- Morgan J, Cannell MGR.** 1988. Support costs of different branch designs: effects of position, number, angle and deflection of laterals. *Tree Physiology* **4**, 303–313.
- Newson RB, Parker JS, Barlow PW.** 1993. Are lateral roots of tomato spaced by multiples of a fundamental distance? *Annals of Botany* **71**, 549–557.
- Nielsen KL, Lynch JP, Jablonski AG, Curtis PS.** 1994. Carbon cost of root systems: an architectural approach. *Plant and Soil* **165**, 161–169.
- Niklas KJ.** 1986. Computer simulations of branching patterns and their implications on the evolution of plants. *Lectures on Mathematics in the Life Sciences* **18**, 1–50.
- Niklas KJ.** 1994. *Plant allometry: the scaling of form and process*. Chicago: University of Chicago Press.
- Niklas KJ, Kerchner V.** 1984. Mechanical and photosynthetic constraints on the evolution of plant shape. *Paleobiology* **10**, 79–101.
- Nye PH, Tinker PB.** 1977. *Solute movement in the soil–root system*. Oxford: Blackwell Scientific.
- Porterfield DM.** 2002. Environmental sensing and directional growth of plant roots. In: Waisel Y, Eshel A, Kafkafi U, eds. *Plants roots: the hidden half*. New York: Marcel Dekker, 471–487.
- Pregitzer KS.** 2002. The fine roots of trees—a new perspective. *New Phytologist* **156**, 267–270.
- Press M.** 1999. The functional significance of leaf structure: a search for generalizations. *New Phytologist* **143**, 213–219.
- Reiss MJ.** 1989. *The allometry of growth and reproduction*. New York: Cambridge University Press.
- Robinson D.** 1994. The responses of plants to non-uniform supplies of nutrients. *New Phytologist* **127**, 635–674.
- Robinson D, Hodge A, Griffiths BS, Fitter AH.** 1999. Plant root proliferation in nitrogen-rich patches confers competitive advantage. *Proceedings of the Royal Society B: Biological Sciences* **266**, 431–435.
- Robinson D, Linehan DJ, Caul S.** 1991. What limits nitrate uptake from soil? *Plant, Cell and Environment* **14**, 77–85.
- Ryzer P.** 1997. The importance of tissue density for growth and life span of leaves and roots: a comparison of five ecologically contrasting grasses. *Functional Ecology* **10**, 717–723.
- Sachs T.** 1991. *Pattern formation in plant tissues*. Cambridge: Cambridge University Press.
- SAS Institute Inc.** 2002–2003. *SAS/STAT user's guide*, Version 9.1.3. Cary, NC: SAS Institute Inc.
- Scott Russell R, Clarkson DT.** 1976. Ion transport in root systems. In: Sunderland N, ed. *Perspectives in experimental biology*. Oxford: Pergamon Press, 405–415.
- Sokal RR, Rohlf FJ.** 1995. *Biometry*, 3rd edn. New York: WH Freeman.
- Taub DR, Goldberg D.** 1996. Root system topology of plants from habitats differing in soil resource availability. *Functional Ecology* **10**, 258–264.
- Valenzuela-Estrada LR, Vera-Caraballo V, Ruth LE, Eissenstat DM.** 2008. Root anatomy, morphology, and longevity among root orders in *Vaccinium corymbosum* (Ericaceae). *American Journal of Botany* **95**, 1506–1514.
- Volder A, Smart DR, Bloom AJ, Eissenstat DM.** 2005. Rapid decline in nitrate uptake and respiration with age in fine lateral roots of grape: implications for root efficiency and competitive effectiveness. *New Phytologist* **165**, 493–501.
- Walch-Liu P, Liu L, Remans T, Tester M, Forde BG.** 2006. Evidence that l-glutamate can act as an exogenous signal to modulate root growth and branching in *Arabidopsis thaliana*. *Plant and Cell Physiology* **47**, 1045–1057.

Zamir M. 1976. Optimality principles in arterial branching. *Journal of Theoretical Biology* **62**, 227–251.

Zar JH. 1984. *Biostatistical analysis* Englewood Cliffs, NJ: Prentice-Hall.

Zhang H, Forde BG. 2000. Regulation of *Arabidopsis* root development by nitrate availability. *Journal of Experimental Botany* **51**, 51–59.

Zhang H, Jennings A, Barlow PW, Forde BG. 1999. Dual pathways for regulation of root branching by nitrate. *Proceedings of the National Academy of Sciences, USA* **96**, 6529–6534.

Zhang N, Hasenstein KH. 1999. Initiation and elongation of lateral roots in *Lactuca sativa*. *International Journal of Plant Sciences* **160**, 511–519.